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14. ABSTRACT

Purpose: To explore two new neuroprotective strategies in the setting of experimental repetitive mild traumatic brain injury (mTBI): i) hyperbaric oxygen (HBO) preconditioning and ii) HBO treatment combined with intranasal delivery of nicotinamide (Vitamin B3). **Scope**: In rat model of a repetitive mild cortical controlled injury, we will investigate the neuropathological profile of two mTBI sessions at two interval times (3, 7d) using non-invasive MRI assessments (T2WI, SWI, and DTI), correlated with histology. The effects of i) a prophylactic HBO strategy (pretreatment 3d X 1hr) prior to first mTBI and ii) the comprehensive therapeutic strategy of 3d X 1hr course of HBO combined with intranasal administration of nicotinamide right after the first mTBI impact will be evaluated using the MRI and histological biomarkers. **Major Findings**: Two sessions of mTBI with 3d apart resulted in significant increase in lesion volumes derived from T2WI and hemorrhagic lesion derived from SWI. Ex vivo histology examination confirms the in vivo neuroimaging findings. HBO pretreatment significantly reduced the lesion volume and hemorrhagic lesion in rmTBI with either 3d or 7d apart. **Progress Report:** There is cumulative neuropathology following repetitive mTBI. The initial mTBI event renders the brain vulnerable to the subsequent mTBI up to 3 days. HBO pretreatment prior to the initial mTBI appears to prevent the detrimental consequences of either single mTBI or repetitive mTBI events.

15. SUBJECT TERMS

Repetitive mild traumatic brain injury, HBO, nitoctinamide, intranasall, MRI, rat

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Neuroprotective Strategies for Repetitive Mild Traumatic Brain Injury (mTBI)

Principal Investigator: Andre Obenaus, Ph.D., Loma Linda University

INTRODUCTION

Mild Traumatic brain injury (mTBI), or concussion, is an important medical concern for the military in the field. Recurrent mTBI episodes may exacerbate tissue injury and psychosocial outcomes (Huh, 2007; Creeley, 2004). In our current study, we hypothesized that repetitive mTBI (rmTBI) would result in cumulative injuries and that intervention using either HBO alone prophylactically or therapeutically in combination with intranasal delivery of nicotinamide would improve the outcomes in a rodent model subjected to rmTBI. Both intervention paradigms are non-invasive, simple to administer and can be rapidly deployed in the field. We proposed the following three Aims: **Aim 1)** To investigate the neuropathological profile of two mTBI events at two interval times (3, 7d), using non-invasive magnetic resonance imaging (MRI) correlated with histology; **Aim 2)** To investigate the effects of a prophylactic HBO strategy (pretreatment) in the setting of rmTBI. The experimental groups (see Aim 1) underwent a 3d X 1hr course of HBO pretreatment followed by mTBI at two interval times (3, 7d). Outcome assessments were as in Aim 1; **Aim 3)** To investigate the comprehensive therapeutic strategy of 3d X 1hr course of HBO and intranasal administration of nicotinamide for three days after the first mTBI impact.

DEVELOPMENT OF AN rmTBI ANIMAL MODEL

We developed a rat model comprised of a mild cortical deformation (0.5 mm) followed by an identical impact (0.5 mm) either at 3 or 7d after the initial injury but at the same site (Fig. 1). Using MRI we evaluated three key parameters: 1) edema development and formation (T2-weighted imaging; T2WI), 2) localization of extravascular blood (susceptibility weighted imaging, SWI); and 3) at the final time point (14d) we evaluated the integrity of white matter using diffusion tensor imaging (DTI). Histology is used to validate the MRI findings and provide a pathophysiological basis for our results. Detailed methods are provided in the Appendix–Huang, 2010, NNS Poster.

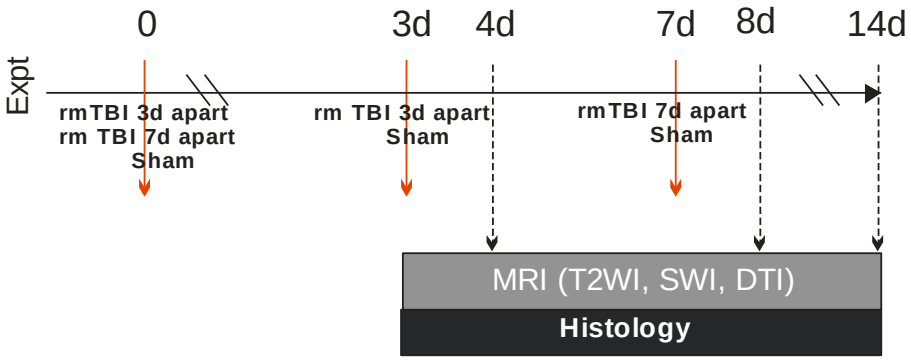

Fig.1 Experimental design for Aim 1. An initial mTBI was induced at day 0, followed by a second repetitive mTBI at day 3 for the rmTBI 3d apart group and at day 7 for group of rmTBI 7d apart group. Sham animals underwent an identical procedure but without CCI. At 24 hrs after each impact and 14 days after the initial mTBI, multi-modal MRI was acquired. Histology was performed at each time point.

Cortical lesion volume, defined as abnormal T2WI signal intensities (hyperintensity = edema, hypointensity = blood) showed significantly increased lesion size in the 3d compared to the 7d rmTBI group at 14 days after the initial impact (Fig. 2). The interval (i.e. 3d vs 7d) between repetitive injury illicits an altered MRI signature. The rmTBI 3d apart group was associated with a heterogeneous mixture tissue types (hypo- and hyperintensities) at the impact site that is composed of both edema and blood. However, there appeared to be little or no edema but only mild bleeding at the injury site when rmTBI is undertaken with a 7d interval

(Fig. 3). The evaluation of blood within tissues can be readily visualized with susceptibility weighted imaging (SWI), a new imaging technique. SWI is uniquely sensitive to the iron content of extravascular blood which can be visualized as dark regions on MR. There was consistent greater SWI derived-hemorrhagic lesion volume in rmTBI with 3d apart (Fig. 4).

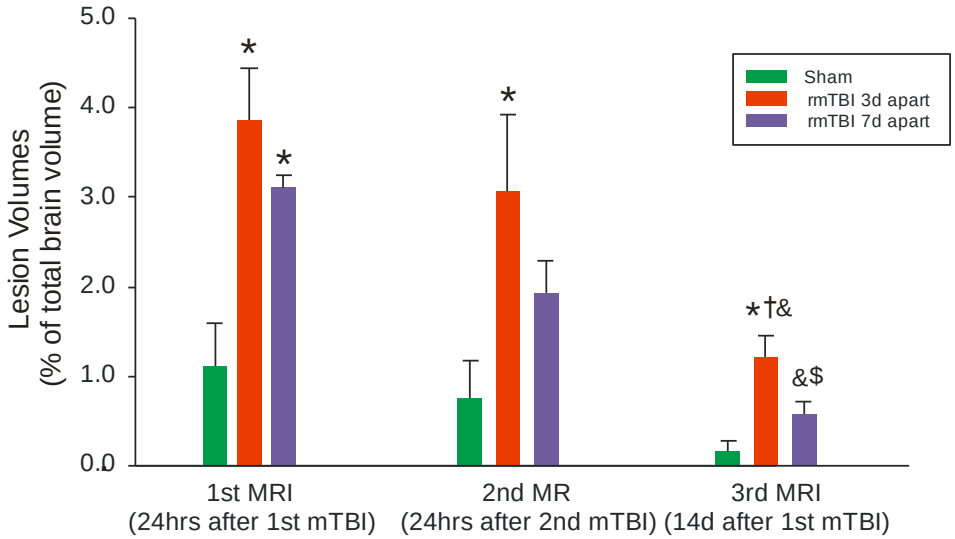

Fig. 2 Lesion volumes derived from T2WI following rmTBI. The first mTBI event resulted in a similar lesion size in both rmTBI 3d and 7d apart groups. When a second mTBI was induced at 3d after the initial impact, there were significant increase in lesion volume compared to Shams and the 7d apart group. *p<0.05 vs Sham; †p<0.05 vs rmTBI 7d apart; $^{\&}$p<0.05 vs 1st MRI; $^{\$}$p<0.05 vs 2nd MRI.

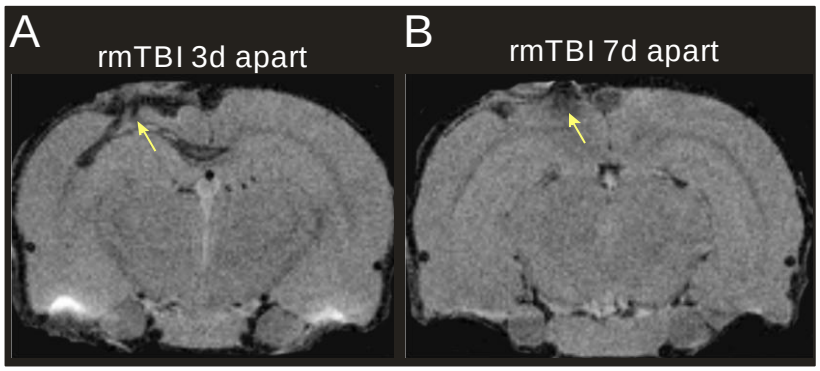

Fig. 3 Representative T2WI showing the signal pattern of the lesion (arrows) in the repetitive mild TBI (rmTBI) groups at 14 days after the initial impact. Hyperintense T2 (bright) is representative of edema formation and hypointensity (dark) indicates blood within the injured brain. A) A heterogeneous signal intensity was evident in animals receiving rmTBI 3 days apart; B) Hypointensities were primarily prominent in the lesion when rmTBI occurred 7 days apart. Also note the smaller lesion area in the rmTBI 7d apart group.

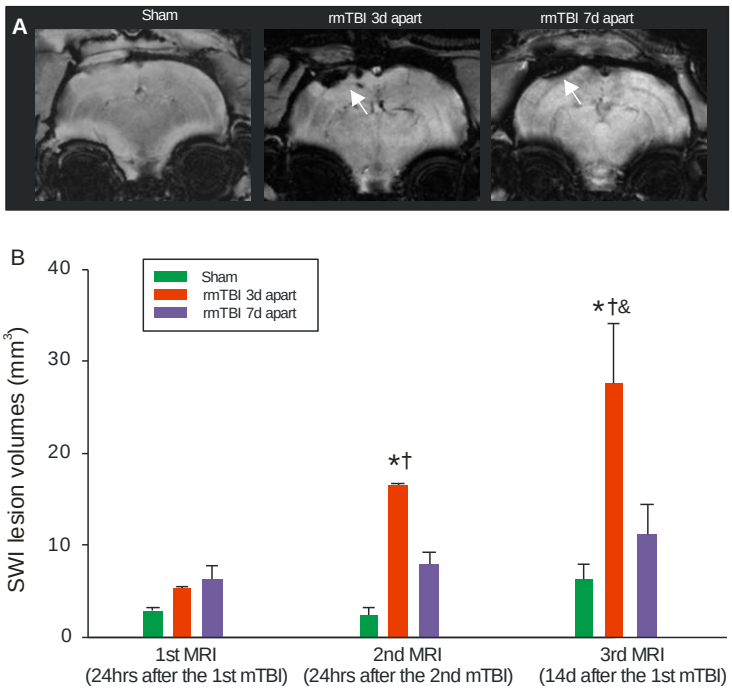

Fig. 4 Hemorrhagic lesion volumes following rmTBI. A) SWI showed the appearance of greater hemorrhage (arrows) in animals who received rmTBI 3d apart compared to 7d apart; B) Quantitative SWI revealed there were significantly increased lesion volumes over 14d when the second mTBI was induced at 3d after the initial impact, which was not observed in the rmTBI 7d apart group. *p<0.05 vs Sham; †p<0.05 vs rmTBI 7d apart group; $^{\&}$p<0.05 vs 1st MRI.

Similar to the T2WI profiles at the final imaging time point, we also observed a significant increase in hemorrhagic lesion volumes at 24hrs after the second injury (Fig.4), suggesting that shorter intervals (3d) between two mTBI events render the brain more susceptible to bleeding.

White matter integrity can be non-invasively assessed using diffusion tensor imaging (DTI), in which water moves preferentially along intact fiber bundles (Obenaus 2007). These data are currently being reviewed and analysis is ongoing.

A serendipitous finding of this model was an observed mismatch between cerebral blood flow (CBF) and metabolism after mTBI as revealed by SWI (See details in Appendix- Barnes S, 2010 NNS Poster). SWI, as noted above, is uniquely sensitive to deoxygenated blood as well as extravascular blood. Thus, deoxygenated blood in vessels (i.e. veins) can be readily observed. We found prominent increase in the number and total vessel length of veins between ipsilateral and contralateral hemispheres after first mTBI in some animals (5 out of 19 rats). This venous asymmetry was not only localized to the area of the impact but was also observed to extend over the entire hemisphere in a rostro-caudal extent (Fig. 5). Longitudinal evaluation (2-3 days later) post injury found that the venous asymmetry was still present (n=2).

The observed venous asymmetry appears to be related to the amount of intracerebral hemorrhage seen on SWI (Fig. 6). Both small and large hemorrhage volumes showed low observed rates of asymmetric veins, whereas moderate hemorrhage showed a very consistent increase in the venous asymmetry. A similar trend was observed with T2 lesion volumes but was not conclusive.

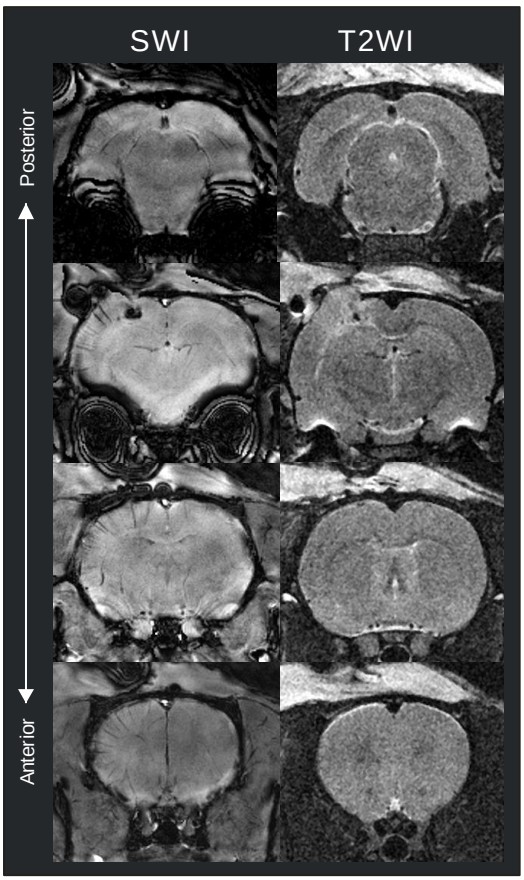

Fig. 5 A unique observation was that using SWI we found a venous asymmetry that extended over the whole hemisphere from anterior to posterior in the injured brain after the 1st impact. Each row represents approximately a 3.3 mm anterior step.

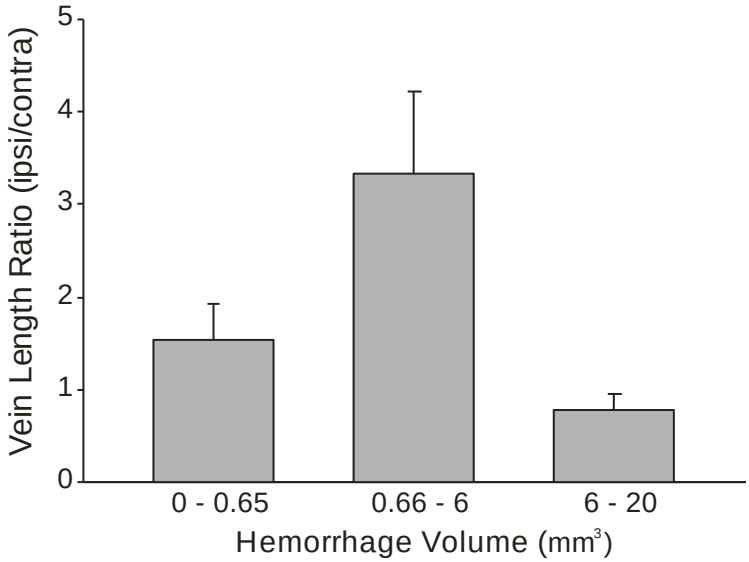

Fig. 6 Hemorrhage volume appeared to influence vein asymmetry. Moderate hemorrhage showed more asymmetry. n=11, 6, and 3, respectively.

These in vivo MRI measures were then validated using histological stains for tissue integrity (cresyl violet, CV) and localization of extravascular blood by staining the presence of iron (Prussian Blue, PB). The lesion size identified by CV staining was correlated to the injury severity as defined by in vivo MRI measures, in which the maximum damage was found in brain tissue that received rmTBI at 3d apart (Fig. 7). Similar to the SWI findings, there was more abnormal iron deposition (blood) in animals subjected to rmTBI which was greater in rmTBI with 3d apart compared to Shams and rmTBI 7d apart group (Fig 8). Using a simple quantification method for PB stain revealed a similar severity to the in vivo SWI findings (Fig. 8). Immunohistochemistry is currently ongoing to examine the changes in neuronal and glial cell within the underlying brain tissue.

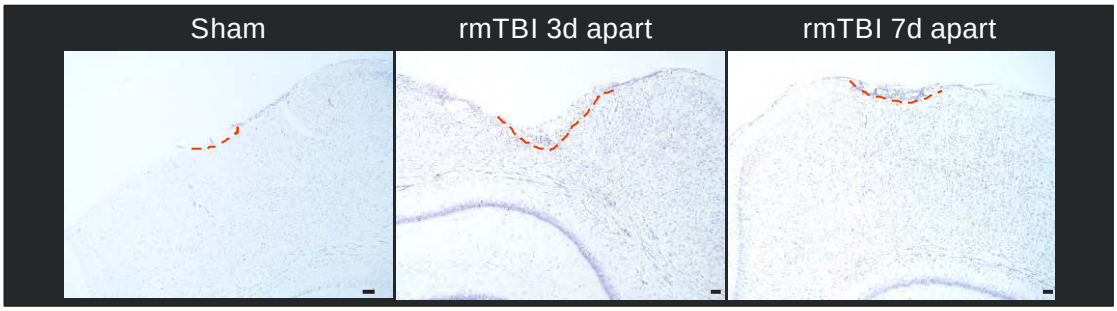

Fig. 7 Cresyl violet (CV) staining at 14 days after the initial mTBI impact. There was an increased lesion area (red dotted line) in 3d apart rmTBI compared to those from the 7d apart group and Shams. These findings were consistent with injury severity assessed by MRI and Prussian blue scoring. Cal bar=100µm

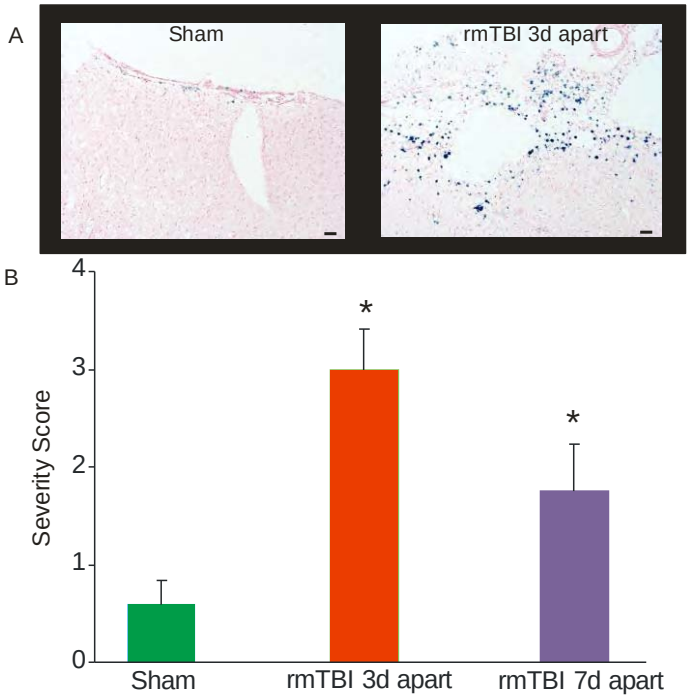

Fig. 8 Prussian blue staining reveled increased iron accumulation at 14 days after the initial mTBI. A) Representative microphotographs showed there was increased Prussian blue staining in brains subjected to rmTBI 3 days apart. Cal bar=100µm; B) Scoring of PB staining confirmed a significant increase in iron deposition within the cortex and corpus callosum of rmTBI groups compared to Shams. Increased iron within tissues was maximum in the rmTBI 3d apart group, suggesting increased hemorrhage and/or bleeding. *p<0.05 vs Sham.

Initial studies evaluating behavioral changes at early times following induction of rmTBI proved to be difficult for a number of reasons including transport to a different building for behavioral testing. In our preliminary studies we found little or no behavioral changes using a variety of measures, including movement initiated escape, turn bias, front and rear paw tape removal tests, open field activity, as well as Morris water maze testing. These preliminary results suggested there were no obvious sensorimotor deficits and cognitive impairments following rmTBI at these early time points. Several reasons for the lack of behavioral effects could include: i) the very mild injury nature generated in our rat model, ii) the relatively short duration after the mTBI event, as previous studies have shown that behavioral deficits are best observed at later time points, and iii) a relatively small sample size. To test long-term behaviorals change, we extended the testing time point to 3 mo after initial mTBI in a select group of animals from rmTBI 3d apart which showed the largest lesion volumes (Fig. 2 and 4). In this small cohort we observed spatial learning deficits using Morris water maze test (Fig.9) in which rmTBI animals swam longer distances to find a submerged platform.

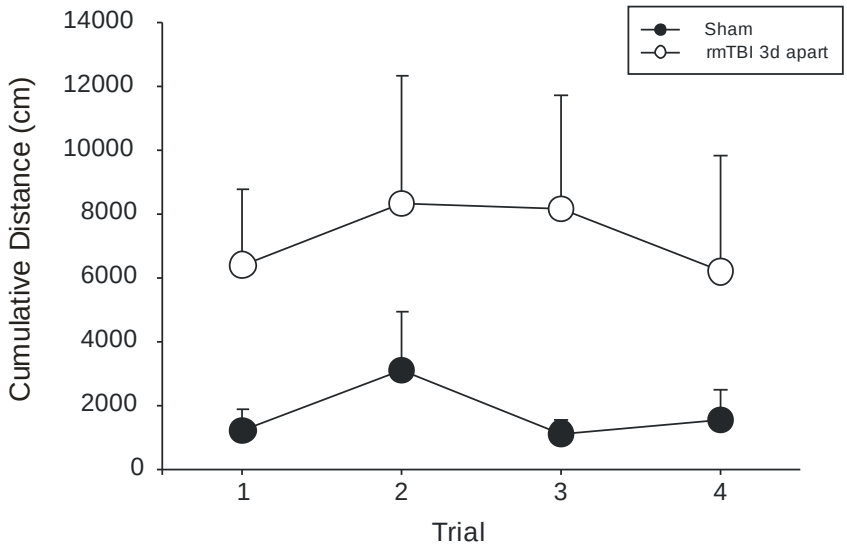

Fig. 9 Morris water maze testing in rmTBI 3d apart animals at 1 mo after the initial impact. rmTBI animals swam for significantly longer distances to find the submerged platform over the 4 trials ((P=0.03), suggesting the impaired spatial learning memory.

These results have been recently presented (Huang, 2010; Barnes, 2010 NNS Poster).Current Status: 90% completed; MRI data analysis is being completed along with histological validation. The neuropathological profiles we will finalize in this rat model of rmTBI will provide a strong valid platform to test our proposed neuroprotective strategies (Aim 2 & 3). Multi-modal MRI is a sensitive monitor for the pathological process in vivo which correlates with histology.

Issues/Limitations: Based on our current progress we have not observed any limitations. In our original proposal we proposed a battery of early neurological and behavioral tests, but we did not observe any significant neurological nor behavioral changes at early post-injury time points. This could suggest that cognitive impairment develops slowly following rmTBI. In future studies, longer time points (months) could be performed for testing the behavioral changes. Indeed, spatial learning deficits were found in a group of rmTBi 3d apart group at 1 mo after the initial impact (Fig. 9).

PROPHALATIC HYPERBARIC OXYGEN PRETREATMENT OF rmTBI:

We initially proposed Hyperbaric Oxygen (HBO) pretreatment to minimize and/or rescue tissues at risk following rmTBI. In our study we treated rats for 1hr/day for 3 consecutive days with 100% oxygen (see methods for details). Previous studies have reported that HBO therapy remains efficacious for up to 12-24hrs after each episode. Using this experimental paradigm we found several key findings (Figs 10, 11, 12):

1) In animals with HBO pretreatment there was reduced tissue abnormalities at the site of impact,

2) Tissue integrity was improved in all HBO animals both after the first mTBI and after second event (either 3 or 7d), and

3) At 14d post rmTBI (our final assessment time point) we observed virtually no loss of tissue nor extravascular blood, in contrast to those seen in tissues without HBO pretreatment.

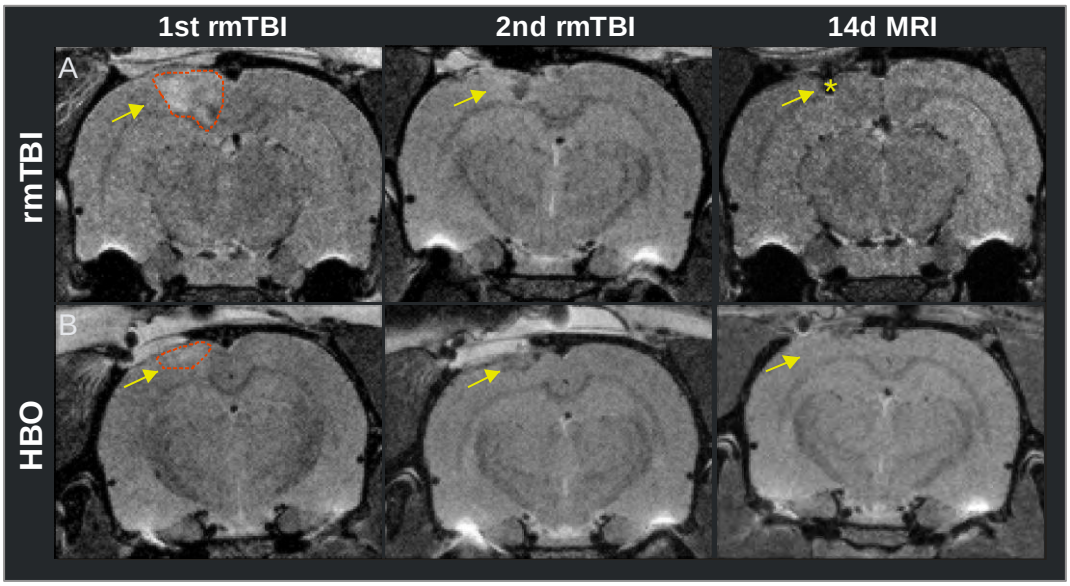

Fig.10 Pretreatment with HBO dramatically rescues tissue at risk following rmTBI. A) rmTBI 3d apart revealed large regions of edema (hyperintensities) with some extravascular blood (hypointensities) on T2WI. Yellow arrows denote the injury site while the red dotted line illustrates the region of tissue abnormality. Over the 14d post-rmTBI there was slow resolution of the edema but significant amounts of extravascular blood remained visible. B) HBO therapy reduced the initial size of the edema (arrow, red dotted line). There were no visible signs of tissue injury after the 2nd rmTBI that was maintained over the 14d time course.

The dramatic ability of HBO to resolve or reduce the amount of extravascular blood within brain tissues is clearly demonstrated in Fig. 11. While there is some blood visible on the SWI images after the 2nd rmTBI, this appears to be primarily on the surface of the brain, compared to blood clearly localized within the brain tissue in rmTBI animals without HBO (Fig 11A).

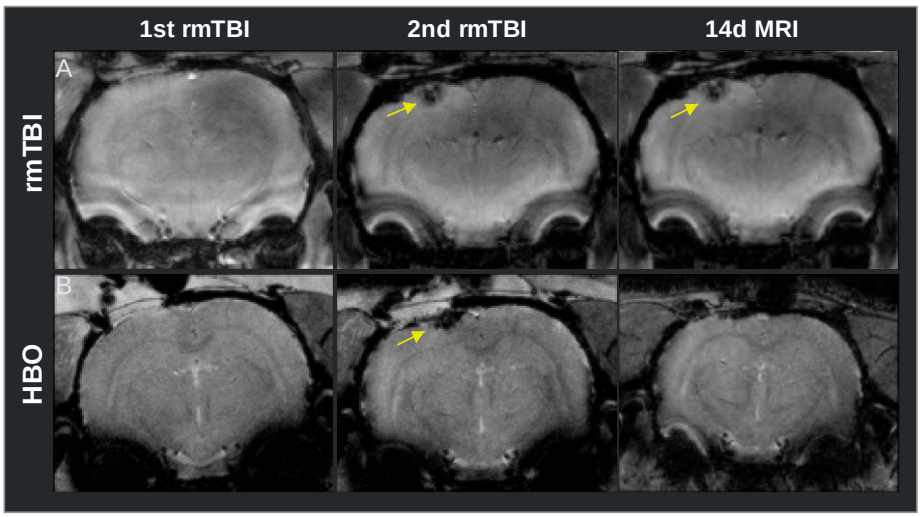

Fig. 11 HBO reduced extravascular blood after rmTBI 3d apart. A) SWI highlighted the location of intra-parenchymal blood after rmTBI which did not resolve over the 14d time course. B) In HBO pretreated animals there was reduced blood visible within the tissues that virtually resolves by 14d.

Quantification of the rmTBI induced lesion volumes at 24hrs after the second hit revealed that there was decreased lesion volume in HBO-treated animals in both the 3d apart and 7d apart groups compared to non-treated animals (Fig. 12A). Similar findings were also found when lesion volume was assessed at 14d (Fig. 12B).

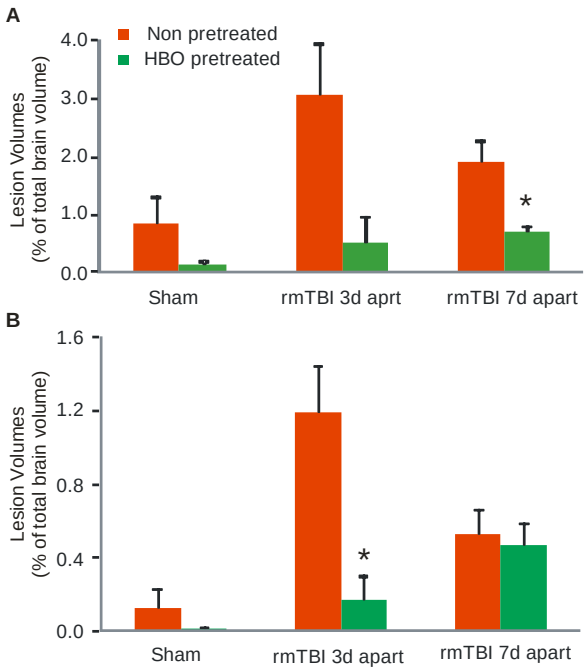

Fig. 12 Lesion volumes derived from T2WI revealed the neuroprotective effects of prophylactic HBO in rmTBI. HBO pretreatment for 3 consecutive days (1h X 3d) prior to rmTBI significantly reduced the lesion volumes at 24hrs after the second mTBI episode (A) and 14d after the initial impact (B). *p<0.05 vs Non-pretreated.

Current Status: The data collection is 60% complete; data collection is concurrent with ongoing data analysis including MRI measures and histology.

Issues/Limitations: Based on our current progress we have not observed any limitations. HBO pretreatment significantly improved the MRI outcomes after rmTBI.

COMBINATORIAL THERAPY (HBO+NICOTIMIDE) IMPROVES rmTBI OUTCOMES

The Aim 3 is currently ongoing.

Current Status: The data collection is 20% completed; data collecting is with ongoing data analysis including MRI measures and histology.

Issues/Limitations: No limitations have been observed to date.

KEY RESEARCH ACCOMLISHMENTS

1. After mTBI, the brain is vulnerable to subsequent mTBI events at 3 days post-initial injury in a rat model of controlled cortical impact.
2. Prophylactic HBO intervention prior to mTBI appears to prevent the detrimental consequences of either single mTBI or repetitive mTBI events.

REPORTABLE OUTCOMES

Abstracts:

1. Huang, L., Coats J., Neglerio K., Mohd-Yusof A., Obenaus A. Temporal alterations in lesion volume in a rat model of repetitive mild traumatic brain Injury. Annual meeting of National Neurotrauma Society, June 14-17, 2010, Las Vegas, Nevada (Poster Presentation)

2. Coats J., Donovan V., Obenaus A.,Huang L. A contra-lateral model of repeated mild traumatic brain injury. Annual meeting of National Neurotrauma Society, June 14-17, 2010, Las Vegas, Nevada (Poster Presentation)

3. Barnes S, Coats J., Huang L., Obenaus A. Mild traumatic brain injury causes unilateral changes in venous blood oxygenation. Annual meeting of National Neurotrauma Society, June 14-17, 2010, Las Vegas, Nevada (Poster Presentation)

4. Coats J. Donovan V., Huang L., Obenaus A. A mild traumatic brain injury is exacerbated by a second contralateral impact. 2010 Army Science Conference.

5. Huang, L. Coats. J, Neglerio K., Mohd-Yusof A., Obenaus A. Repetitive mild traumatic brain injury in the rat results in increased lesion volume. 2010 Army Science Conference.

CONCLUSIONS

A mild TBI event renders the brain vulnerable to subsequent mild TBI exposure. HBO has the potential for an effective neuroprotective strategy that can be applied to victims at high risk for repetitive mTBI.

REFERENCES

1. Huh JW, Widing AG, Raghupathi r. Basic science; repetitive mild non-contusive brain trauma inimmature rats exacerbates traumatic axonal injury and axonal calpain activation: a preliminary report. J Neurotrauma 2007;24:15-27.

2. Creeley CE, Wozniak DF, Bayly PV, Olney JW, Lewis LM. Multiple episodes of mild traumatic brain injury result in impaired cognitive performance in mice. Acad Emerg Med 2004;11:809-819.

3. Obenaus A, Robbins M, Blanco G, et al. Multi-modal magnetic resonance imaging alterations in two rat models of mild neurotrauma. J Neurotrauma 2007;24:1147-1160.

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Temporal Alterations in Lesion Volume in A Rat Model of Repetitive Mild Traumatic Brain Injury

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Introduction

Repetitive mild traumatic brain injury (mTBI) is an important medical concern for active military personnel with 10-20% reporting neurological or psychological symptoms. Given that insignificant or transient neurological effects after a single mTBI event may easily go unreported, it is hypothesized that a second mTBI event could be exacerbated by the effects of the initial event. Thus, multiple mTBI events could result in cumulative brain injury, leading to exacerbation of tissue damage and psychosocial outcomes. In the present study, we characterized the neuropathological profiles of repetitive mTBI using a rat model where the first mild impact was then followed by a second mTBI at intervals of 1, 3, 7 days using non-invasive magnetic resonance imaging (MRI), correlated with histology.

Methods

Twenty Sprague Dawley adult male rats (2 mo old) were randomized into 4 groups with two episodes of mTBI: 1) 1 day apart; 2) 3 days apart; 3) 7 days apart; and 4) Shams. Study design was shown in Fig.1.

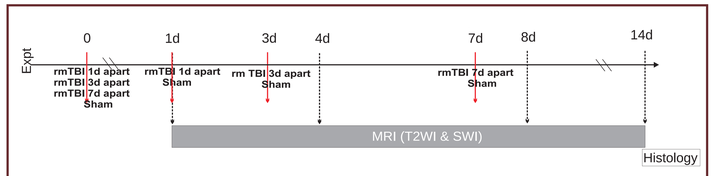

Fig.1 The first mTBI was induced at day 0, followed by the second repetitive mTB at day 1 for group of rmTBI 1d apart, at day 3 for group of rmTBI 3d apart and at day 7 for group of rmTBI 7day apart, respetively. Sham animals will undergo the identical procedure except for the delivery of impact injury. At 24 hrs after each impact and 14 days after the initial mTBI, MRI including T2 weighted imaging (T2WI) and susceptibility weighted imaging (SWI) was acquired. Ex vivo histology was performed after the final MRI.

Rat model of repetitive mTBI

Rats were anesthetized with isoflurane (3% induction, 2% maintenance) and the head fixed onto a rat stereotaxic frame. After incision of the skin, a 4.5 mm craniotomy was performed at 4 mm posterior and 3.5 mm lateral to bregma. Care was taken to prevent disturbing the underlying dura and minimizing bleeding at the craniotomy site. A mild controlled cortical impact (CCI) was delivered using an electromagnetic driven piston (0.5 mm depth, 4 mm diameter tip at 6.0 m/s, 200 ms dwelling duration). A second identical impact was delivered at 1, 3 or 7 days after the first CCI event at the same location. For all animals, body temperature was maintained at 37±1°C with a heating pad. Randomized sham animals underwent the same surgical procedure without CCI. After surgery, the wound was sutured and Bupronex (0.01mg/kg, im) was administrated to minimize pain and discomfort. Animals were placed in warmed box for recovery after which they were returned to their cages.

MRI data collection and analysis

At 24 hr after each CCI and 14 days after the initial impact, rats were lightly anesthetized using isoflurane (3% induction, 1% maintenance) and body temperature maintained at 37 ±1 ºC using a thermostat controlled heated water cushion. MR data was collected on a Bruker Advance 4.7T MRI with quadrature coil (Bruker Biospin, Billerica MA). A T2WI sequence was acquired with 3453ms/20ms/20 of TR/TE/Flip angle, 3 cm of field of view (FOV), 25 slices. An SWI sequence was also acquired with 39ms/20ms/20 of TR/TE/Flip angle, 3 cm of FOV and 48 slices.

Using Cheshire imaging processing software (Hayden Image/Processing Group, Waltham, MA), the lesion volumes were obtained (including abnormal both hyper- and hypo-intensity).

SWI magnitude and phase data were post-processed for high pass filter phase and SWI images using in-house software (Spin). The lesion hypointensity was drawn semi-automatically within cortex and subcortical white matter on each slice and manually checked. Total hemorrhagic volumes were calculated over all slices.

Histology

Prussian blue staining (PB) and Cresyl violet (CV) staining were performed on 4% PFA fixed brain tissues to evaluate the abnormal iron deposition and cell death, respectively.

To quickly quantify PB staining, a simple score was assessed on the slice at the level with maximum lesion size. A score of 1 was counted whenever the PB was positive in areas of ipsilateral or contralateral cortex/subcortical white matter. The highest score was 4. A higher score suggests a severer injury.

Statistics

One-way ANOVA analysis was performed to statistically examine quantitative measures.

Abbreviations

rmTBI = Repetitive mild traumatic brain injury
MRI = Magnetic Resonance Imaging
T2WI = T2 weighted imaging
SWI= Susceptibility weighted imaging
CCI= Controlled cortical impact

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Results

All animals survived the CCI injury induction.

MRI assessments

1. T2WI lesion volume

There were no significant differences in lesion volumes among the three mTBI groups after 24 hrs following the 1st TBI event. However, in the repetitive mTBI groups of 1 and 3 days but not 7 days apart, a second CCI resulted in increased T2WI lesion volumes that persisted until 14 days (Fig. 2).

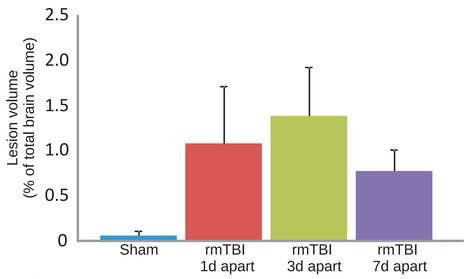

Fig. 2 T2WI-derived final lesion volumes at 14 days after an initial mild TBI episode. rmTBI resulted in a cumulative injury with increased severity after the second mTBI delivered at 1 and 3 days apart compared to 7 days apart and Sham.

2.T2WI signal pattern within lesion (Fig. 3)

Fig. 3 Representative T2 image slices showing the lesion signal pattern (arrows) in the three rmTBI groups at 14 days after the initial impact. While hyperintense T2 (bright) suggests the edema formation, the hypointensity (dark) indicates blood in the injured brain. A) Hyperintensities were prominent within the lesion when two mTBI were given 1 day apart; B) A heterogeneous signal intensities were evident in animals receiving rmTBI 3 days apart; C) Hypointensities were prominent in the lesion when rmTBI occurred 7 days apart.

3. SWI lesion

SWI revealed that rmTBI resulted in hemorrhage which was maximal in rats receiving a second mTBI at 3 days apart (Fig. 4 & Fig. 5).

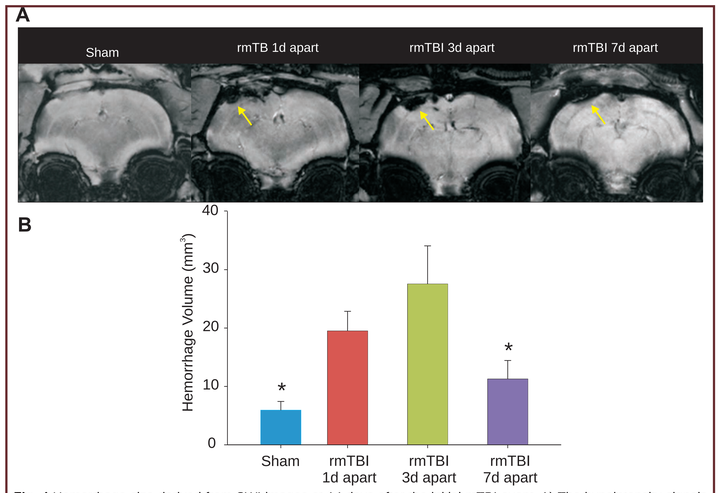

Fig. 4 Hemorrhage size derived from SWI images at 14 days after the initial mTBI event. A) The hypointensity signals (arrows) in the representative SWI image slices indicated hemorrhage within the traumatized cortex. There was greater hemorrhagic lesion in brains receiving rmTBI at 1 or 3 days apart; B) Quantitative SWI-derived hemorrhage lesion volumes were significantly greater in rats subjected to rmTBI at 3 days apart compared to other rmTBI intervals. These SWI findings are consistent with histological results (PB staining) where in the underlying brain tissue revealed more iron accumulation. *p<0.05 vs rmTBI 3d apart.

Histology

1. Prussian blue staining (Fig. 5 & 6)

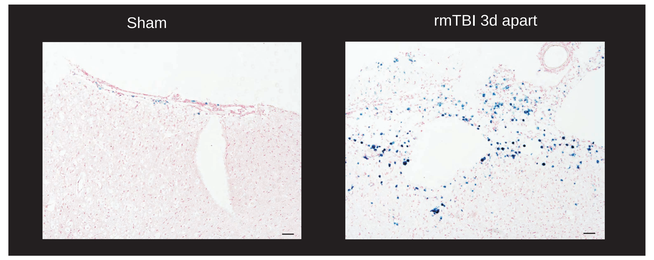

Fig. 5 Representative microphotographs of Prussian blue staining. In comparison to Sham, there was increased Prussian blue staining in brains subjected to rmTBI 3 days apart, suggestive of increased hemorrhage. Cal bar=100µm

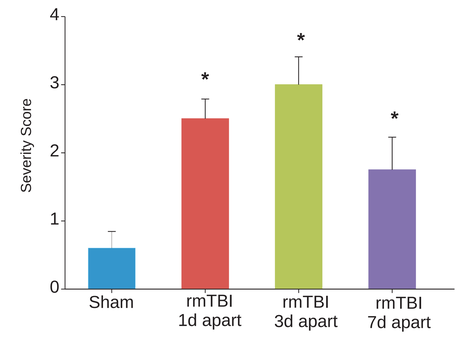

Fig. 6 Scoring of Prussian blue stained sections reveled increased iron accumulation assessed at 14 days after the initial mTBI. rmTBI resulted in significant greater iron deposition (bleeding) within the cortex and corpus callosum compared to Sham. Increased iron within tissues was maximum in the rmTBI 3d apart group. *p<0.05 vs Sham.

2. Cresyl violet staining confirmed the brain was vulnerable to the repetitive mTBI episode up to 3 days after the initial mTBI (Fig. 7). The histological results were consistent to MRI findings in vivo.

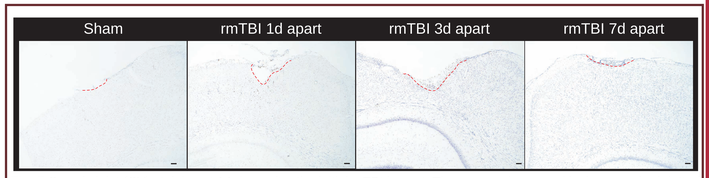

Fig. 7 Cresyl violet (CV) staining at 14 days after the initial mTBI impact. There was a increased lesion area (red dotted line) in subjects of rmTBI with either 1 or 3 days apart, compared to those of 7 days apart and Sham. These findings were consistent with injury severity assessed by MRI and Prussian blue scoring. Cal bar=100µm

Conclusions

Our novel findings suggest that the brain appears to exhibit heightened vulnerability to a second mild traumatic insult up to 3 days after an initial mild TBI event. In addition, early secondary TBI appears to progress from edema (1 day apart) to enhanced bleeding at later time points (7 days), but the rmTBI 3 days apart results in maximal cumulative injury with lesion composed of edema and blood components. Thus, the temporal evolution of repetitive mTBI of the underlying neuropathology should be reflected by appropriate treatment strategies. Rat models of repetitive mTBI may serve as a clinically relevant platform for investigation into injury-induced neurostructural deficits, and provide the basis for evaluation of outcome parameters for testing experimental therapeutics. We have also demonstrated that MRI is a sensitive neuroimaging biomarker for monitoring the pathological evolution after repetitive mTBI that can be rapidly translated into the military medical clinic.

Mild Traumatic Brain Injury Causes Unilateral Changes in Venous Blood Oxygenation

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Introduction

Susceptibility weighted imaging (SWI) magnetic resonance imaging (MRI) is sensitive to levels of deoxygenated hemoglobin and blood products. SWI is therefore ideally suited to image the venous vasculature and hemorrhage and is commonly used in animal and human studies[1]. We evaluated a novel finding where Sprague Dawley rats that were subjected to a mild traumatic brain injury (mTBI) from a single controlled cortical impact (CCI) were noted to have increased numbers of prominent veins on the ipsilateral brain hemisphere. Increased vascular contrast could indicate less oxygenated venous blood (increased amounts of deoxygenated hemoglobin), enlarged veins, or both.

We hypothesized that even a very mild injury can cause changes in oxygen delivery or utilization by the brain tissue.

Results

A prominent increase in the number of veins was observed between ipsilateral and contralateral hemispheres in seven of 19 animals, five mTBI and two shams. The two shams that showed the vein asymmetry likely had moderate disruptions of the cortical surface during the craniotomy, but as no T2 images were collected on these two sham animals this could not be confirmed.
The venous asymmetry was not localized to the area of the impact but was observed to extend over the entire hemisphere (Figure 2).
Longitudinal evaluation (2-3 days later) post injury found that the venous asymmetry was still present (n=2).
The venous asymmetry appears to be related to the amount of intracerebral hemorrhage seen on SWI (Figure 4). Both small and large hemorrhage volumes showed low observed rates of asymmetric veins, whereas moderate hemorrhage showed a very consistent increase in the venous asymmetry. A similar trend was observed with T2 lesion volumes but was not conclusive.

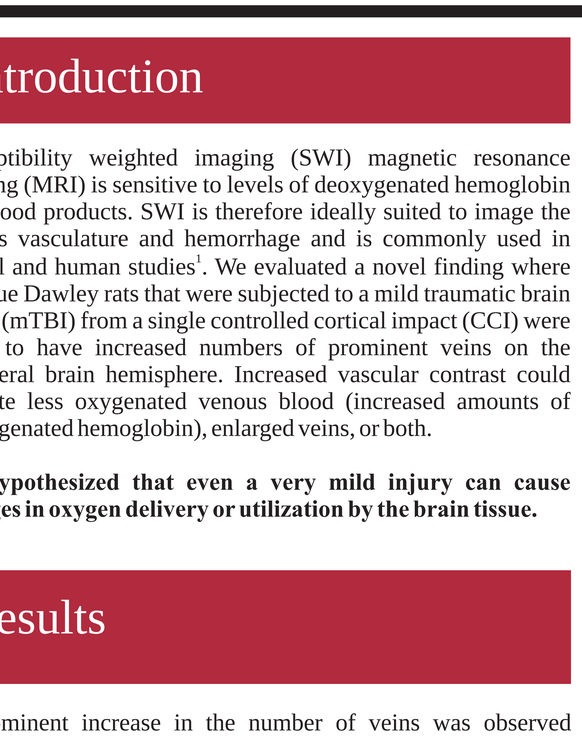

Figure 1. The left hand column shows SWI images and the right hand column shows the corresponding T2 images, each row is a different animal. The top two animals show clear venous asymmetry while the bottom two do not. A) and B) show a mTBI animal with venous asymmetry and hemorrhage at the site of CCI impact (arrows). C) and D) show a mTBI animal with venous asymmetry slightly anterior to the site of CCI impact. E) and F) show a mTBI animal with hemorrhage but no venous asymmetry at the site of CCI impact (arrows). G) and H) show a control with no venous asymmetry at the site of the craniotomy.

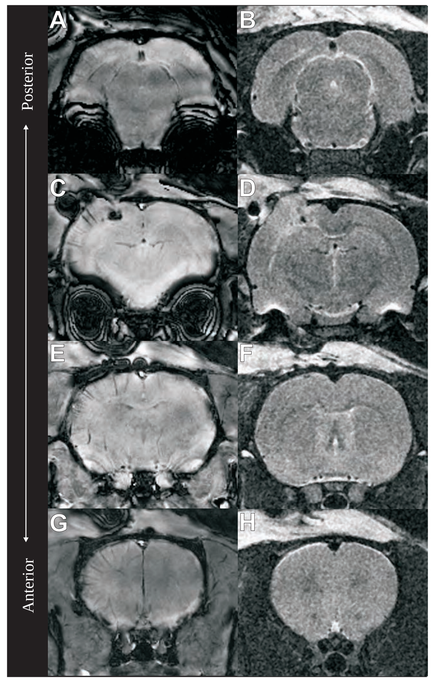

Figure 2. The venous asymmetry extends over the whole hemisphere from anterior to posterior. A) and B) are the most posterior and each row represents approximately a 3.3 mm anterior step. The left column is SWI and the right column is T2.

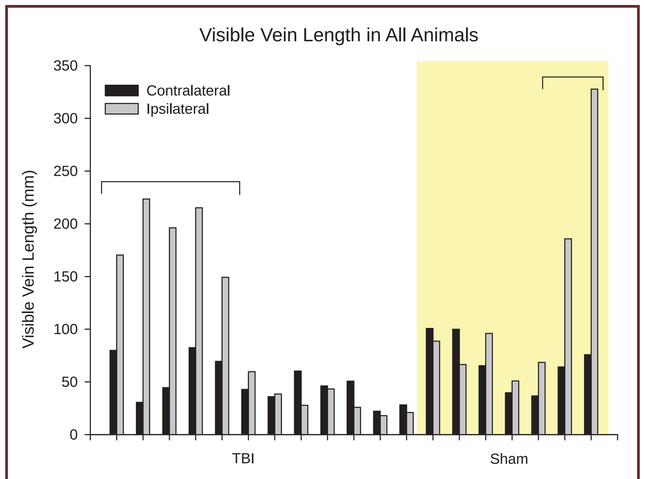

Figure 3. Cumulative vein length was increased in five TBI and 2 sham animals (n=19 total) which showed an abnormal prominence of veins on the ipsilateral side compared to the contralateral side. This effect can be triggered by even a very mild injury (i.e. shams).

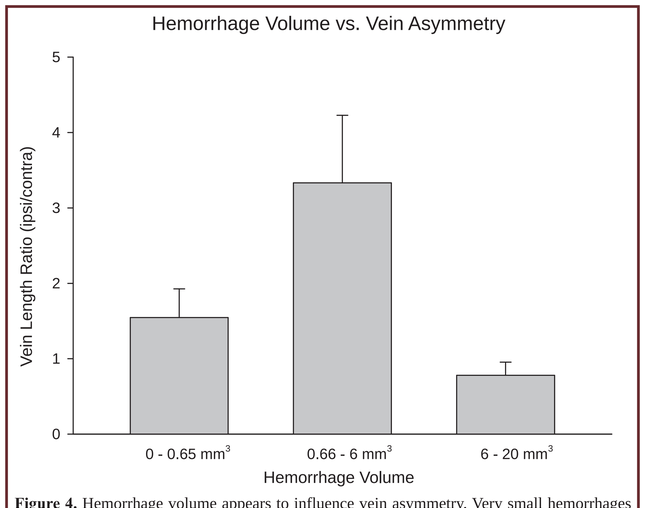

Figure 4. Hemorrhage volume appears to influence vein asymmetry. Very small hemorrhages show some vein asymmetry, moderate hemorrhage shows asymmetry more reliably, and large lesions do not appear to show asymmetry at all (n=11, 6, and 3, respectively).

References:
1. Haacke E, et al., 2009. Susceptibility-weighted imaging: technical aspects and clinical applications, part 1. AJNR 30, 19-30.
2. Kelly DF, et al., 2000. Ethanol Reduces Metabolic Uncoupling Following Experimental Head Injury. J Neurotrauma 17, 261-272.

The two sham animals that showed a vein asymmetry likely exhibited minor cortical disruptions during the craniotomy. The remaining shams (no apparent cortical injury) establish a consistent baseline with a normal number of visible veins and therefore a normal amount of deoxygenated hemoglobin. The mTBI animals can then be clearly divided into two groups, one that shows a vein asymmetry and significantly more veins then the shams, and one that shows significantly less veins then the shams. This suggest that the cerebral blood flow (CBF) and metabolism are uncoupled after mTBI and can result in either abnormally high or low levels of deoxygenated hemoglobin. This uncoupling has been reported previously in a more severe injury model[2]. A group of naive controls without a craniotomy is required to clearly establish this.
In a select group of mTBI rats followed with PET revealed hypo-metabolism in the area of increased venous contrast at 24 hours.

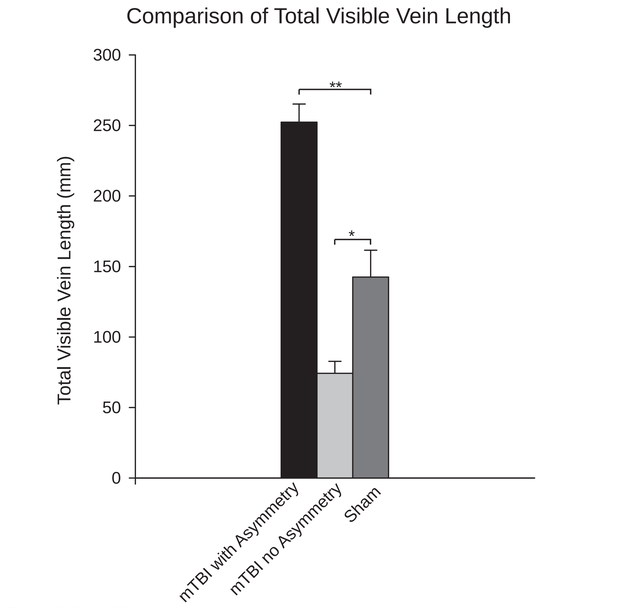

Figure 5. The mTBI group can be divided into two groups: a group with asymmetric veins, and a group without asymmetric veins. Both of these groups had significantly different amounts of visible veins compared to shams. * $p<0.05$, ** $p<0.01$

Methods

Nine shams and 12 mTBI Sprague Dawley rats were initially evaluated, two shams were eliminated due to a moderate injury observed with SWI or T2 resulting in 7 shams. A 5 mm craniotomy was performed on all animals, immediately followed by a mild 3 mm diameter CCI injury only on the mTBI animals: 6m/s velocity, 200ms dwell time, and 0.5 mm depth. SWI was performed on all animals and T2 weighted imaging was performed on 13 of these animals 24 hours after impact, immediately followed by 18-FDG positron emission tomography (PET) on seven of the animals (four shams, three TBI). MRI parameters were TR/TE/FA/Matrix/Averages = 39ms/20ms/20°/256x256x48/4 for the SWI and 3560ms/20-70ms/90°/256x256x25/4 for the six echo T2. All images were acquired on a Bruker 4.7T horizontal bore magnet. SWI images were processed using standard techniques with a 48x48 high pass filter using our own in house software (SPIN, Detroit, MI). The length of all visible veins on SWI images were measured to get a total visible vein length for each hemisphere, only cortical veins were considered. Hemorrhages were manually drawn on SWI images to get hemorrhage volumes, and lesion volume was manually drawn on the T2 images.

Conclusions

The venous mismatch is likely a result of an uncoupling of metabolism from cerebral blood flow (CBF) that has been previously observed following severe TBI[2]. The distinct two groups of mTBI suggests that this uncoupling can result in abnormally high or low levels of deoxygenated hemoglobin. Changes to both metabolism and flow are likely responsible for this uncoupling. These preliminary results need to be extended to examine the implications and underlying mechanisms.

However, non-invasive SWI can offer a method to identify and study the mismatch between CBF and metabolism after mTBI.

Abbreviations

CCI - Controlled Cortical Impact
CBF - Cerebral Blood Flow
FA - Flip Angle
FDG - Flurodeoxyglucose (18F)
MRI - Magnetic Resonance Imaging
mTBI - Mild Traumatic Brain Injury
PET - Positron Emission Tomography
SWI - Susceptibility Weighted Imaging
TR - Repetition Time
TE - Echo Time

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